

TECHNICAL COMMENTS

Comment on "Failure of Bone Marrow Cells to Transdifferentiate into Neural Cells in Vivo"

Castro *et al.* (1) reported that bone marrow cells (BMCs) fail to generate neural cells in vivo. The bone marrow they injected came from mice in which LacZ expression was driven by a widely expressed trapped promoter. In principle, such cells and their progeny should readily be detected by staining for β -galactosidase activity. To validate their method, Castro *et al.* presented two brain sections, one completely white (transplanted mouse) and one completely blue (donor). The problem with these results, however, is that blue LacZ-positive microglia—which, like other monocyte/macrophage cells, originate from hematopoietic stem cells [see (2)]—were absent from the brains of the transplanted animals.

In contrast, several investigators have shown that BMCs expressing enhanced green fluorescent protein (EGFP) under the control of a similarly widespread promoter give rise to EGFP-positive microglia in the brains of transplanted animals (3–5). Nakano *et al.* (6) and Eglitis and Mezey (7) obtained similar results using a retroviral insert or the Y chromosome, respectively, as markers of cell lineage.

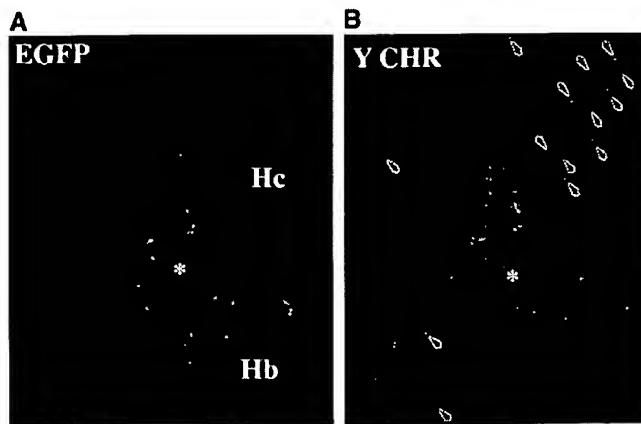
One might argue that Castro *et al.* did not wait long enough to see tagged microglia, but approximately 6% of the microglial cell population is replaced per month (3, 8). Thus, they should have detected LacZ in 6 to 24% of the microglia present in the brains of their animals, equivalent to 500 to 1000 positive cells per section at the shortest time (1 month) studied.

Their failure to detect LacZ-positive cells indicates that either their staining method failed or that the LacZ transgene was not expressed in their samples.

We noticed a similar result in irradiated female mice transplanted with EGFP-expressing male marrow cells. There were many more Y chromosome-positive cells than green cells in the brain, even when we used a very sensitive immunohistochemical technique to visualize EGFP (Fig. 1). In peripheral tissues (thymus, spleen, or bone marrow) we detected many bright green cells, most of which were also Y chromosome-positive (when the nucleus was in the section). In view of these results, we question the conclusions in (1), though we agree that the demonstration of bone marrow-derived cells in the brain surely "depend[s] on the experimental system in which the hypothesis is tested." The system used by Castro *et al.* (1) may not have been an ideal one.

The use of protein products of transgenes as markers to follow graft fate is plagued with problems, but these may not have been emphasized enough. It is virtually impossible to achieve ubiquitous transgene expression. Transgenes, including those driven by the Rosa26 promoter, suffer from instability in several tissues (9). Detection of low levels of LacZ is also difficult, and is sensitive to fixation and staining conditions (9). Furthermore, reporter expression might place cells at a growth disadvantage compared with cells without a reporter construct.

Fig. 1. Discrepancy between EGFP expression and the presence of Y chromosome in the CNS after gender mismatched EGFP bone marrow transplantation. Coronal brain sections of a female mouse that received a male EGFP bone marrow transplant one month earlier are shown. (A) EGFP-derived fluorescence (FITC filter). Except for a few cells in the choroid plexus (asterisk), there are no green cells visible. (B) Photograph of the same area using a rhodamine (red) fluorescence filter. Labeled Y chromosomes appear as red dots (arrows). Note the numerous red dots in the brain parenchyma (cortex and habenula) and the lack of green cells in the same regions. Hb, habenula; Hc, hippocampus.



Combined with instability of the transgene, selection against labeled cells could make the vast majority, if not all, of the grafted cells "invisible." Undoubtedly, reporters make grafting experiments less tedious to perform, but because of the present uncertainties concerning transgenic expression tagging, DNA markers such as Y chromosomal probes are far more reliable.

We recently showed that bone marrow-derived cells enter the brain and differentiate into neural cells in humans (10) and that they give rise to cheek epithelial cells without any evidence of fusion (11). A similar report confirmed that mouse BMCs can indeed become neural cells and showed that this happens without fusion (5). Thus, we feel that adult stem cells may be induced to reconstitute other tissues than those from which they were harvested.

Éva Mezey

National Institute of Neurological Disorders and Stroke
Building 36, Room 3D-10
9000 Rockville Pike
Bethesda, MD 20892, USA
E-mail: mezeye@ninds.nih.gov

Andras Nagy

Mount Sinai Hospital
Samuel Lunenfeld Research Institute
600 University Avenue
Toronto, ON M5G 1X5, Canada

Ildiko Szalayova

Sharon Key

National Institute of Neurological Disorders and Stroke

Andras Bratincsak

National Institute of Mental Health
Bethesda, MD 20892, USA

Judit Baffi

National Eye Institute
Bethesda, MD 20892, USA

Tal Shahar

National Institute of Neurological Disorders and Stroke

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